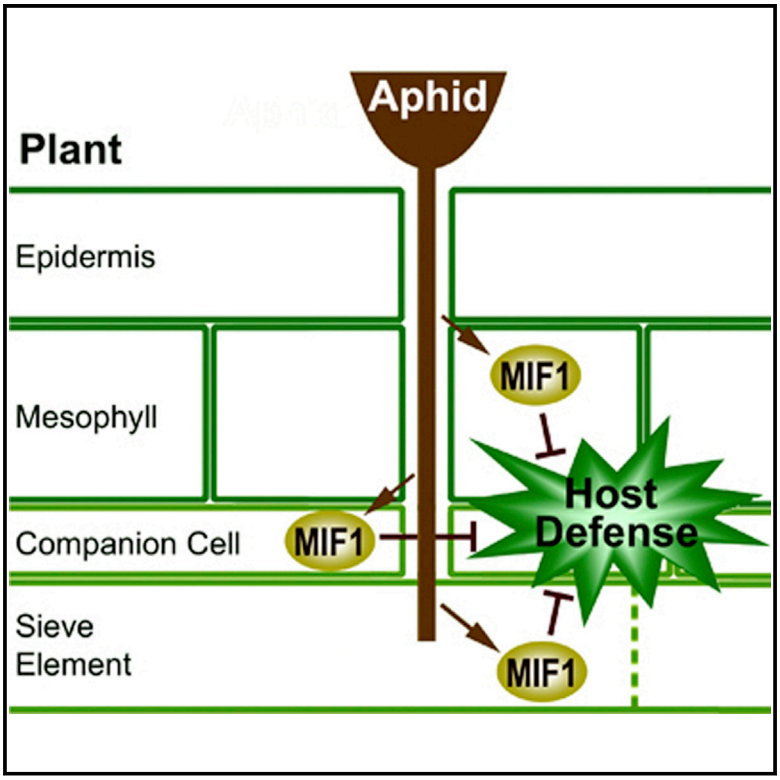
Report

Current Biology

A Secreted MIF Cytokine Enables Aphid Feeding and Represses Plant Immune Responses

Graphical Abstract



Authors

Elodie Naessens, Géraldine Dubreuil, Philippe Giordanengo, ..., Naïma Minet-Kebdani, Harald Keller, Christine Coustau

Correspondence

harald.keller@sophia.inra.fr (H.K.), christine.coustau@sophia.inra.fr (C.C.)

In Brief

Naessens et al. demonstrate that a MIF cytokine is secreted in aphid saliva during feeding. They show that this protein inhibits major plant immune responses and is crucial to aphid infection of a host plant. This suggests a so-far-unsuspected conservation of infectious processes between parasites of animal and plant species.

Highlights

- A macrophage migration inhibitory factor (MIF) is secreted in aphid saliva
- Expression of aphid MIFs in planta inhibits plant immune responses
- Expression of aphid MIF1 is necessary for aphid survival and feeding on a host plant

Accession Numbers

KP218519
KR136352
KR136353

Naessens et al., 2015, Current Biology *25*, 1898–1903
July 20, 2015
http://dx.doi.org/10.1016/j.cub.2015.05.047

CellPress

CellPress

Current Biology
Report

A Secreted MIF Cytokine Enables Aphid Feeding and Represses Plant Immune Responses

Elodie Naessens,[1,3] Géraldine Dubreuil,[1,3,4] Philippe Giordanengo,[1,2] Olga Lucia Baron,[1] Naïma Minet-Kebdani,[1] Harald Keller,[1,*] and Christine Coustau[1,*]

[1]INRA, Université Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France
[2]Université de Picardie Jules Verne, 33 rue Saint Leu, 80039 Amiens, France
[3]Co-first author
[4]Present address: Institut de Recherche sur la Biologie de l'Insecte, UMR 7261, CNRS/Université François-Rabelais, Parc Grandmont, 37200 Tours, France
*Correspondence: harald.keller@sophia.inra.fr (H.K.), christine.coustau@sophia.inra.fr (C.C.)
http://dx.doi.org/10.1016/j.cub.2015.05.047

SUMMARY

Aphids attack virtually all plant species and cause serious crop damages in agriculture [1]. Despite their dramatic impact on food production, little is known about the molecular processes that allow aphids to exploit their host plants. To date, few aphid salivary proteins have been identified that are essential for aphid feeding, and their nature and function remain largely unknown [2–4]. Here, we show that a macrophage migration inhibitory factor (MIF) is secreted in aphid saliva. In vertebrates, MIFs are important pro-inflammatory cytokines regulating immune responses [5, 6]. MIF proteins are also secreted by parasites of vertebrates, including nematodes, ticks, and protozoa, and participate in the modulation of host immune responses [7–9]. The finding that a plant parasite secretes a MIF protein prompted us to question the role of the cytokine in the plant-aphid interaction. We show here that expression of *MIF* genes is crucial for aphid survival, fecundity, and feeding on a host plant. The ectopic expression of aphid MIFs in leaf tissues inhibits major plant immune responses, such as the expression of defense-related genes, callose deposition, and hypersensitive cell death. Functional complementation analyses in vivo allowed demonstrating that MIF1 is the member of the MIF protein family that allows aphids to exploit their host plants. To our knowledge, this is the first report of a cytokine that is secreted by a parasite to modulate plant immune responses. Our findings suggest a so-far unsuspected conservation of infection strategies among parasites of animal and plant species.

RESULTS AND DISCUSSION

Aphids Secrete a MIF1 Salivary Protein

We previously showed [10] that the pea aphid *Acyrthosiphon pisum* expresses five members of a *MIF* multigene family (*ApMIF1–ApMIF5*; Figure 1A). Here, we show the existence and expression of three *MIF* members in the green peach aphid *Myzus pericicae*, which we named *MpMIF1* (GenBank: KP218519), *MpMIF3* (GenBank: KR136352), and *MpMIF4* (GenBank: KR136353) according to their sequence similarities with the *A. pisum* MIFs [10]. Sequence similarities among aphid MIFs are more pronounced between identical members from different species (orthologs) than between different members of the same species (paralogs; Figure S1). This is consistent with previous results showing a complex evolutionary history, which is characterized by differential gene loss and duplication across insect phyla and which occurred before the diversification of aphids [10].

In both *A. pisum* and *M. persicae*, transcripts of all *MIF* genes were detected in whole bodies (Figures 1A and 1B). However, *ApMIF1* and *MpMIF1* were the unique members to be expressed in salivary glands (Figures 1A and 1B). The presence of MIF peptides in aphid saliva was recently reported in a proteomic study [12]. Here, MIF proteins were detected in artificial medium after feeding of aphids (Figure 1C), demonstrating that both species secrete the cytokine during feeding.

Secretion of MIF proteins has been reported for a variety of vertebrate host-infecting parasite species, including nematodes, such as hookworms [11]; protozoa, such as *Leishmania* and *Plasmodium* [7]; and ticks [8, 13]. Several studies provided evidence that parasite MIFs participate in host immunomodulation [11, 14]. The molecular mechanisms underlying interference with the host immune system remain to be elucidated for most of these vertebrate-parasite interactions, but hookworms likely operate through the endogenous MIF-related signaling pathways of the host [11]. The secretion of a MIF by aphids is intriguing, as it raises the question of the role of such cytokine in the aphid-plant interaction.

Expression of Aphid MIFs Is Required for an Exploitation of the Host Plant

To investigate whether expression of *MIF1* affects the success of the aphid-plant interaction, we used RNAi of *MIF1* on the model aphid *A. pisum.* Analysis of the interference efficiency and dynamics showed that, despite the sequence differences between genes of the *MIF* family (Figure S1), the RNAi targeting of *MIF1* results in a strong under-expression of the three well-expressed *ApMIF*s, *ApMIF1*, *ApMIF2*, and *ApMIF3* (Figure S2). This non-specific effect of dsRNA on related genes (orthologs or paralogs)

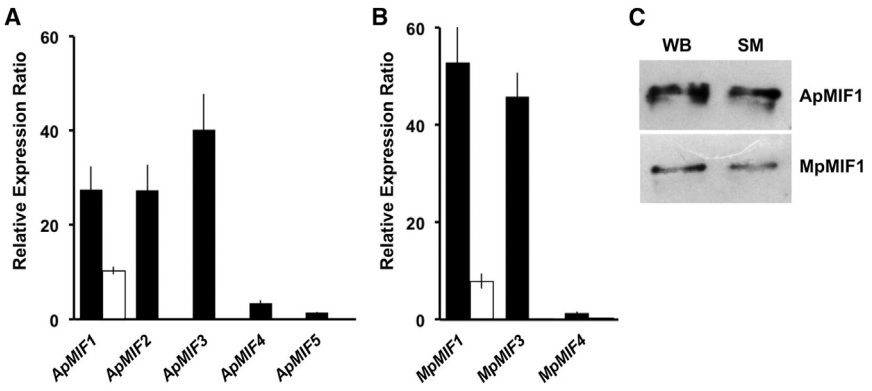


Figure 1. Expression of Aphid MIF1 in Salivary Glands

(A and B) Relative expression ratios of MIF members in whole bodies (black) and salivary glands (white) from (A) the *A. pisum* genetic lineage LL01 and (B) *M. persicae*. Expression values are normalized to *EF1* expression and shown as a percent of total expression of MIFs in whole bodies. Each bar represents the mean expression ± SD obtained from three independent experiments.

(C) Representative western blots showing ApMIF1 or MpMIF1 protein in 15 μg total protein prepared from aphid whole bodies (WB) or saliva-conditioned medium (SM). Antibodies were raised against two MIF1 peptides [11] (Figure S1).

has been previously reported and shown to function on genes containing as few as 11 contiguous nucleotides identical to the triggered transcript [15]. Under-expression of *ApMIF*-encoding genes results in a significant decrease in survival and fecundity of *A. pisum* when feeding on their host plant *Vicia faba* (Figure 2A). By contrast, no difference in survival and fecundity was observed between *MIF1*-dsRNA injected and control aphids, when maintained on artificial feeding medium (Figure 2B). Therefore, the reduced survival and fecundity of aphids that under-express *MIF*s seem not to result from a general deleterious effect of MIF repression but rather from an impediment of the interaction with the host plant. We thus analyzed in more detail the feeding behavior of aphids that under-express *MIF*s. Electropenetrography (EPG) recordings (Figures 2C and 2D) showed no variation in the pathway phases (phase 1 and 2) between *GFP*- and *MIF*-dsRNA-injected aphids (Figure 2C; Table S2). By contrast, aphids that under-express *MIF* showed significantly reduced periods of long-repeated punctures in phloem elements, required more time for initiating phloem sap ingestion, and remained for a much-shorter time in the phloem feeding phase 3 (Figure 2E). This altered behavior decreased the overall ratio of aphids that succeeded in phloem feeding (Table S1).

The EPG parameters show that the repression of *MIF* genes does not affect the aphid's ability to explore plant tissues but rather disturbs their ability to feed from phloem sap. Long-repeated punctures realized in companion cells and phloem cells are a prerequisite for phloem feeding [16], and alteration in these punctures has been reported for aphids being exposed to resistant plants [17]. Taken together, our results show that the aphid requires MIF proteins for exploiting the plant and suggest that these cytokines may play a role in modulating the defense responses of the host.

Aphid MIFs Interfere with the Plant Immune System

To analyze whether aphid MIF proteins interfere with immune responses of the host plant, we used the *Agrobacterium-tumefaciens*-mediated transient expression system to produce MIFs in *Nicotiana benthamiana* leaves. This versatile method allows delivering genes into the plant cell nucleus for functional analysis and is commonly used for analyzing the interference of parasite- and pathogen-derived proteins with plant immune responses [18]. As *A. pisum* is unable to proliferate on *N. benthamiana*, we used sequences from the aphid *Myzus persicae*, which is adapted to *N. benthamiana* as a host. To analyze the effect of aphid MIFs on plant defenses, we stimulated the plant immune responses with the elicitor cryptogein, a 10-kDa protein from the oomycete plant pathogen, *Phytophthora cryptogea* [19]. When applied to plants from the genus *Nicotiana*, cryptogein elicits multifaceted defense responses, including those governed by salicylate- and jasmonate-dependent signaling pathways. This leads to the production of pathogenesis-related (PR) proteins [19], the synthesis of callose for cell wall reinforcement [20], and, ultimately, to plant cell death as a visible outcome of the cryptogein-induced hypersensitive response (HR) in *Nicotiana* [19]. As expected, cryptogein induced the cell death response in *N. benthamiana* leaves upon local application (Figure 3A). By contrast, cell death did not occur in leaves that produced the aphid proteins (Figure 3A), indicating that *MpMIF1*, *MpMIF3*, and *MpMIF4* repress the cryptogein-induced HR. The transfection with *MpMIF*s alone or with an empty vector control did not result in visible symptoms (Figure 3A). The inhibitory effect of MIFs on apoptosis in animal cells is well documented [21], and our results show an analogous effect of aphid MIFs on the hypersensitive cell death of plant cells.

Callose deposition is an efficient host response to injury by insect pests or pathogens [3, 22]. *N. benthamiana* leaves showed strong callose synthesis upon cryptogein treatment (Figure 3B). This response was repressed in cryptogein-treated leaves producing MpMIF proteins, and callose levels were similar to those observed in non-elicited leaves (Figure 3B). Congruently, MpMIFs also impaired the cryptogein-induced transcriptional activation of the *N. benthamiana PR* genes, *NbPR1*, *NbPR2*, and *NbPR3* (Figure 3C). Overexpression of genes encoding the acidic NbPR1 and NbPR2 proteins characterizes the activated salicylate hormone-signaling pathway, whereas an induction of the gene encoding basic NbPR3 is associated with jasmonate-dependent defenses [19]. The MpMIFs thus appear to prevent immune responses, which are mediated by both hormones. It has to be noted that the MpMIFs also induced callose apposition and *PR* gene expression to some extent in control (cry −) *N. benthamiana* leaves (Figures 3B and 3C). Dual activities in activating and suppressing host defense responses were also shown for the salivary *M. persicae* protein of unknown function, Mp10 [3, 23].

Taken together, the data show that aphid MIFs inhibit defense responses when delivered to plant cells. To our knowledge, this

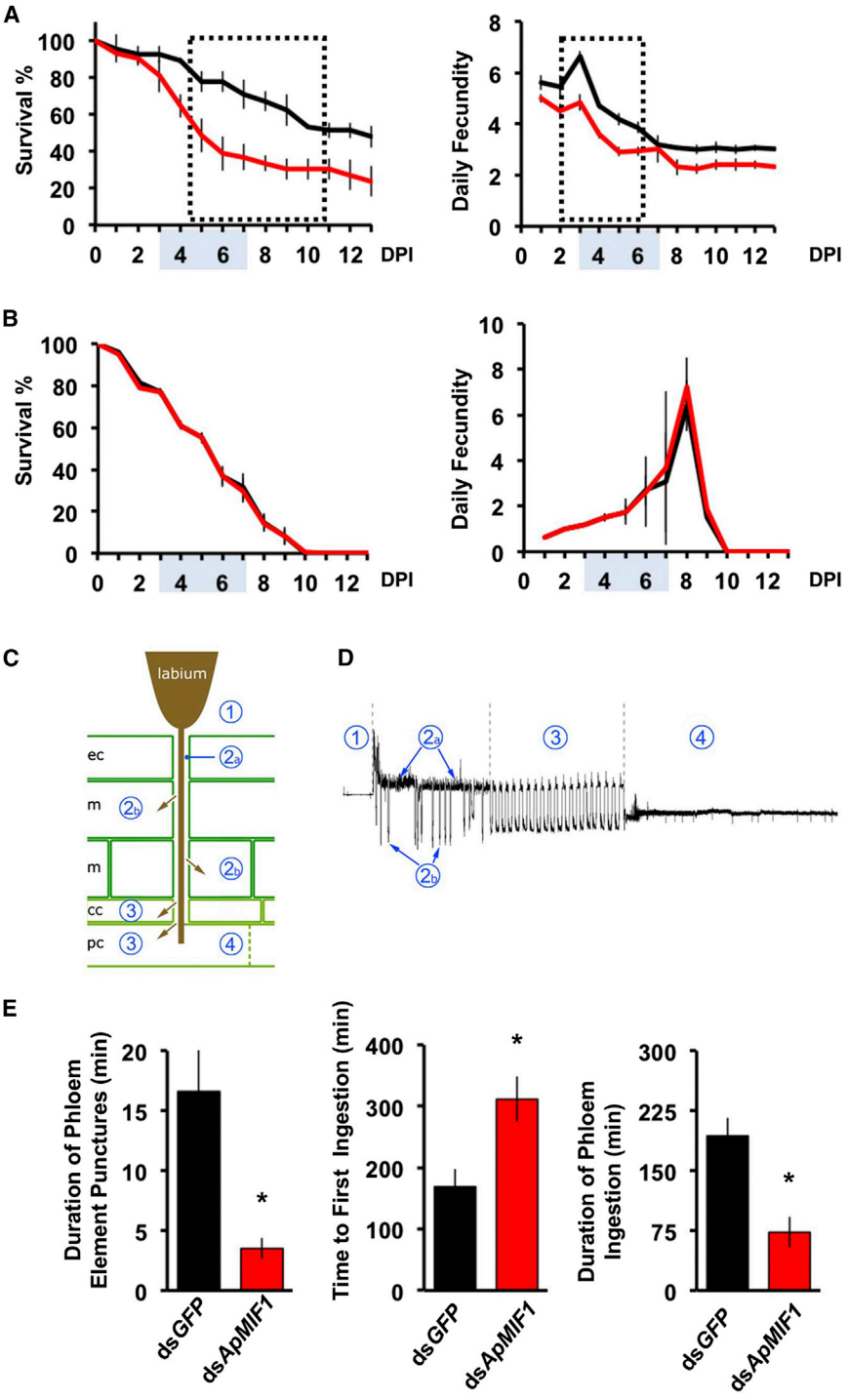


Figure 2. MIFs Are Required for an Exploitation of the Host Plant

(A and B) Survival rate (%) and average individual offspring production (daily fecundity expressed as number of offspring per day per adult) of adult *A. pisum* aphids feeding on plants (A) or maintained on artificial diet (B) for 13 days post-injection (DPI) of dsRNA. Aphids were injected with non-relevant *GFP* dsRNA (black lines) or *ApMIF1* dsRNA (red lines). Highly significant differences between *GFP* dsRNA- and *ApMIF1* dsRNA-injected aphids are boxed with a dashed line ($p < 0.001$). The blue box on the x axis corresponds to the period of optimum RNAi efficiency (see Figure S2). Data are means ± SD from triplicate experiments carried out on 20 individuals.

(C) Schematic representation of aphid stylet progression within plant tissues during feeding. After a non-probing period (1) aphid stylet progression toward the phloem cells is achieved through insertion between epidermic cells (ec) (2a), followed by punctures of mesophyll cells (m) (2b), long-repeated punctures of companion cells (cc) and their associated phloem cells (pc) (3), and finally, insertion in the phloem cells (pc) for phloem sap ingestion (4).

(D) Reconstructed typical electropenetrogram showing the corresponding feeding phases.

(E) Comparison of EPG parameters from the feeding phases 3 and 4 in *GFP* dsRNA- (ds*GFP*; black bars) or *ApMIF1* dsRNA- (ds*ApMIF1*; red bars) injected aphids. Error bars represent the SE of triplicate experiments carried out on 24 individuals. The asterisks indicate differences with $p < 0.05$ according to the Mann-Whitney U-test. All parameters are listed in Table S1.

represents the first evidence that animal cytokines interfere with plant immune functions and raises the question of the nature of the plant-signaling pathways that are targeted by aphid MIFs. In mammals, signal transduction pathways engaged by MIFs are complex and still not fully characterized. MIFs are known as multifunctional molecules operating as cytokines and as enzymes [5, 6]. Extracellular MIF may undergo endocytosis and bind to the intracellular protein JAB-1, which results in a down-regulation of MAPK signals. Alternatively, MIFs can bind the extracellular receptor CD74 and form a MIF-CD74-CD44 complex, which activates the ERK/MAPK pathways. However, they can also bind to and signal through the G-protein-coupled chemokine receptors CXCR, thus activating integrins and calcium influxes [24–26]. In addition to these complex physiological activities, MIFs act as enzymes with D-dopachrome tautomerase, phenylpyruvate tautomerase, and thiolprotein-oxidoreductase activities [26]. The question of the molecular activities and partner molecules of MIFs in non-mammalian species was previously raised in a study involving a MIF protein from a gastropod snail [27]. Although lacking important elements of mammalian MIF signal transduction pathways such as CD74 immune receptors, the snail MIF was shown to present the expected activities of induction of immune cell proliferation, inhibition of p53-mediated apoptosis, and to be involved in the snail anti-parasitic response [27]. The mode of action of MIFs in plants is even more intriguing, because specialized cells of the immune system are missing and because immune responses such as

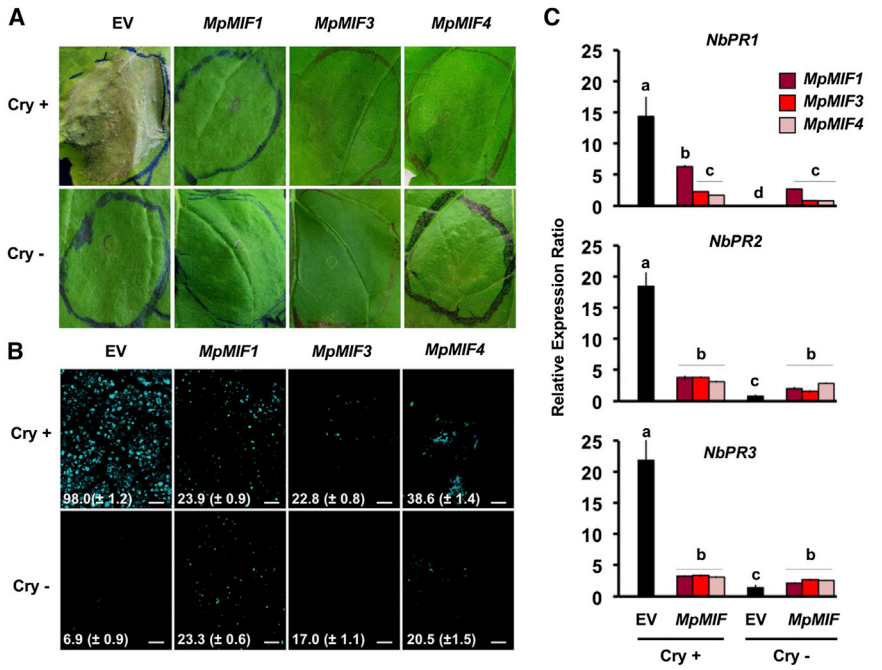


Figure 3. MpMIFs Impair Plant Immune Responses

(A and B) Representative pictures showing hypersensitive cell death (A) and callose deposition (B) in *N. benthamiana* leaves. Leaves were inoculated with *A. tumefaciens* harboring the empty vector (EV) or the *MpMIF* constructs (*MpMIF1*, *MpMIF3*, or *MpMIF4*) 24 hr prior to infiltration with cryptogein (Cry+) or water (Cry−). The efficiency and dynamics of *Agrobacterium*-mediated *MpMIF* expression in planta are shown in Figure S3. (A) The visible symptoms of cryptogein-induced hypersensitive cell death are dehydration and brown lesions (top left). These symptoms do not establish in leaf areas that express *MpMIF*s. The local expression of *MpMIF*s in leaf tissues does not trigger hypersensitive cell death (lower lane) neither does the empty vector control (bottom left). Photographs were taken 48 hr after inoculation with *A. tumefaciens* and 24 hr after treatment with cryptogein or water. (B) Callose deposition is induced by cryptogein in infiltrated leaf areas and appears as blue fluorescence after aniline blue staining (top left). Cryptogein-induced callose deposition is reduced in leaf areas that express *MpMIF1*, *MpMIF3*, or *MpMIF4* to levels that are triggered by the local expression of *MpMIF* genes in the absence of cryptogein. Agroinfiltration with the empty vector control (bottom left) did not induce callose deposition. Photographs were taken 36 hr after inoculation with *A. tumefaciens* and 12 hr after treatment with cryptogein or water. The scale bars represent 150 μm. Numbers indicate means ± SD of callose spots obtained for six individual leaf discs. (C) Expression of the defense-related genes *NbPR1*, *NbPR2*, and *NbPR3* in leaf areas transfected with the EV (black bars) or *MpMIF*s (gradation of red bars) prior to treatment with cryptogein (Cry+) or water (Cry−). Expression of the target genes is presented as a relative expression normalized to internal reference genes and to expression in the control sample (EV/Cry−). Bars represent means ± SD from three biological replicates. Values marked by different letters are significantly different ($p < 0.001$; ANOVA and Tukey-Kramer test). Values marked by identical letters are not significantly different.

callose deposition and defense hormone signaling are plant specific. Understanding the mode of action of MIFs in plants will definitely deserve future complex and dedicated studies.

It has to be noted that the plant immune-suppressive activity of the aphid cytokines appears to be conserved among different members of the MIF protein family, including those (such as MpMIF3 and MpMIF4) that are not expressed in salivary glands. Therefore, we investigated whether impaired survival and fecundity of *MIF*-silenced, plant-feeding aphids result from synergistic effects between the different MIF proteins or from the specific reduction of MIF1 protein in salivary secretions.

MIF1 Is Sufficient and Required for Aphid Feeding on the Host Plant

The *M. persicae-N. benthamiana* model was used to confirm the role of MIF members in vivo. Similar to what we observed for *A. pisum*, RNAi-mediated interference of *MIF1* expression resulted in a downregulation of the two well-expressed MIF genes in *M. persicae* (Figure S4). This downregulation significantly decreased the survival and fecundity of aphids when maintained on their host plant (Figure 4A) but had no effect when aphids fed on artificial medium (Figure 4B). The downregulation of *MIF* gene expression thus specifically influences the capacity of both *M. persicae* and *A. pisum* to interact with their host plant. A complete recovery of normal survival and fecundity rates was obtained when *MIF*-downregulated (RNAi) aphids were fed on *N. benthamiana* ectopically expressing *MpMIF1* (Figure 4C). A partial recovery was observed when aphids were fed on *N. benthamiana* expressing *MpMIF3* (Figure 4D) or *MpMIF4* (Figure 4E). This partial recovery is likely due to the functional redundancy among MIF proteins and their ability to inhibit plant defense responses (Figure 3). However, only MIF1 proteins are secreted in aphid saliva, and a complete functional complementation of survival and fecundity is achieved only by expressing *MpMIF1* in plants.

Taken together, we show that aphids express three (*M. persicae*) to five (*A. pisum*) *MIF* genes. Expression profiles indicate that the cytokines modulate the various biotic interactions that aphids undergo with endosymbiotic bacteria, parasites, and pathogens [11]. Here, we show that aphids dedicate one particular cytokine from the MIF repertoire (MIF1) to their salivary glands, thus providing a mean to interfere with the immune system of host plants. We suggest that aphids commutated a regulatory element from their immune system, which originally participates in warding off parasites, into a device for improving their own parasitic activity.

Further studies have to show whether the cytokines that are secreted by animal and plant parasites interfere with analogous signaling cascades in host cells. It has to be noted that genes encoding MIF cytokines were recently described in the model plant, *Arabidopsis thaliana* [28]. Furthermore, we identified four genes in the *N. benthamiana* genome that code for cytokines and have similarities with the MpMIF1 protein (Figure S5). It is therefore possible that MIFs secreted by aphids mimic or antagonize host proteins to repress plant immune responses. Cytokine mimicry was shown for the tick *Amblyomma americanum*,

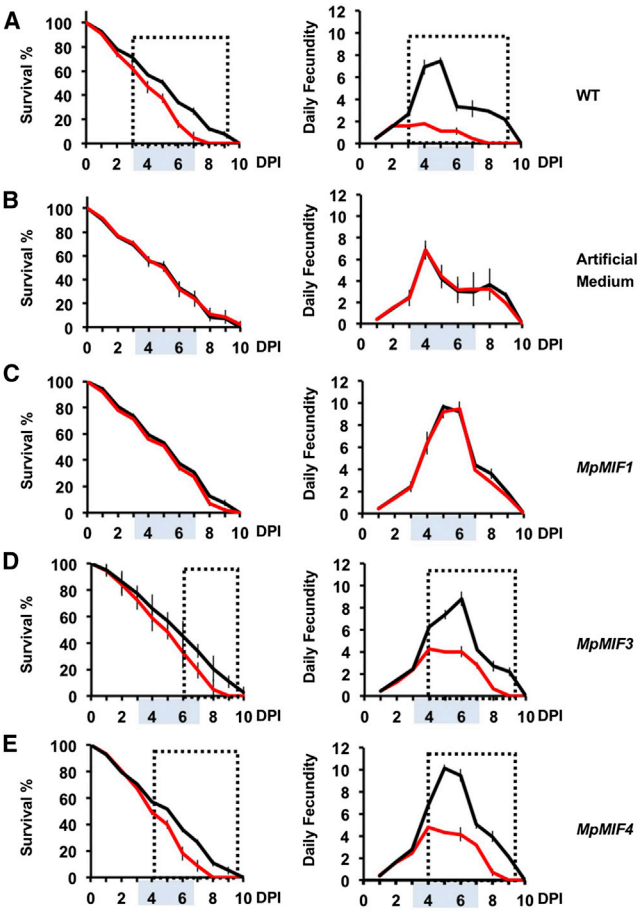


Figure 4. Host-Delivered MIF1 Restores Lifespan and Fecundity of Cytokine-Silenced Aphids

Survival rate (%) and average individual offspring production (daily fecundity expressed as number of offspring per day per adult) of adult *M. persicae* aphids feeding on non-transformed *N. benthamiana* leaves (A); maintained on artificial diet (B); or feeding on *N. benthamiana* leaves expressing *MpMIF1* (C), *MpMIF3* (D), or *MpMIF4* (E) for 10 days post-ingestion (DPI) of dsRNA. Highly significant differences between *GFP* dsRNA- (black lines) and *MpMIF1* dsRNA-silenced aphids (red lines) are boxed with a dashed line ($p < 0.001$; Kruskal-Wallis test). The blue box on the x axis corresponds to the period of optimum RNAi efficiency (see Figure S3). Data are means ± SD from triplicate experiments carried out on 50 individuals.

which secretes a salivary MIF into animal hosts to protect the parasite from being repelled [29].

This first report of a parasite cytokine allowing plant exploitation via salivary secretion suggests a so-far-unsuspected conservation of particular infectious processes between parasites of animal and plant species.

EXPERIMENTAL PROCEDURES

Biological material and procedures for the characterization of MpMIFs; reverse transcription real-time quantitative PCR (qRT-PCR); aphid maintenance on artificial diet; the detection of secreted aphid MIFs; plasmid design for *Agrobacterium*-mediated transformation; transient expression in *N. benthamiana*; analysis of plant immune responses and RNAi; analysis of aphid feeding behavior, survival, and fecundity; and statistical analyses are specified in the Supplemental Experimental Procedures.

ACCESSION NUMBERS

The accession numbers for the three *MIF* members reported in this paper are GenBank: KP218519 (*MpMIF1*), GenBank: KR136352 (*MpMIF3*), and GenBank: KR136353 (*MpMIF4*).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.05.047.

AUTHOR CONTRIBUTIONS

E.N. designed and performed experiments involving plants and *M. persicae*, G.D. designed and performed experiments involving *A. pisum*, P.G. performed the EPG analyses, O.B. participated in the RNAi experiments, N.M.-K. participated in the development of *N. benthamiana* studies, and C.C. and H.K. conceived and supervised the study and drafted the manuscript. All authors approved the final manuscript.

ACKNOWLEDGMENTS

We thank Benoit Industri for providing the cryptogein preparation and Olivier Migliore for growing all the plants necessary for this study. Authors are grateful to the numerous colleagues from the Institut Sophia Agrobiotech who contributed by fruitful discussions. Image acquisitions were performed on the Microscopy Platform-Sophia Agrobiotech Institut-INRA 1355-UNS-CNRS 7254-INRA PACA-Sophia Antipolis. This work was supported by the French Laboratory of Excellence initiatives "SIGNALIFE" (ANR-11-LABX-0028-01), INRA, and CNRS. E.N. was supported by the French Ministry for Education, Research, and Technology.

Received: December 2, 2014
Revised: April 23, 2015
Accepted: May 26, 2015
Published: June 25, 2015

REFERENCES

1. Kim, C.S., Schaible, G.D., Garrett, L., Lubowski, R.N., and Lee, D.J. (2008). Economic impacts of the U.S. soybean aphid infestation: a multi-regional competitive dynamic analysis. Agric. Resource Econ. Rev. *37*, 227–242.
2. Mutti, N.S., Louis, J., Pappan, L.K., Pappan, K., Begum, K., Chen, M.-S., Park, Y., Dittmer, N., Marshall, J., Reese, J.C., and Reeck, G.R. (2008). A protein from the salivary glands of the pea aphid, *Acyrthosiphon pisum*, is essential in feeding on a host plant. Proc. Natl. Acad. Sci. USA *105*, 9965–9969.
3. Bos, J.I.B., Prince, D., Pitino, M., Maffei, M.E., Win, J., and Hogenhout, S.A. (2010). A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). PLoS Genet. *6*, e1001216.
4. Elzinga, D.A., De Vos, M., and Jander, G. (2014). Suppression of plant defenses by a *Myzus persicae* (green peach aphid) salivary effector protein. Mol. Plant Microbe Interact. *27*, 747–756.
5. Calandra, T., and Roger, T. (2003). Macrophage migration inhibitory factor: a regulator of innate immunity. Nat. Rev. Immunol. *3*, 791–800.
6. Bucala, R. (2000). Signal transduction. A most interesting factor. Nature *408*, 146–147.
7. Augustijn, K.D., Kleemann, R., Thompson, J., Kooistra, T., Crawford, C.E., Reece, S.E., Pain, A., Siebum, A.H.G., Janse, C.J., and Waters, A.P. (2007). Functional characterization of the *Plasmodium falciparum* and *P. berghei* homologues of macrophage migration inhibitory factor. Infect. Immun. *75*, 1116–1128.
8. Bowen, C.J., Jaworski, D.C., Wasala, N.B., and Coons, L.B. (2010). Macrophage migration inhibitory factor expression and protein localization in *Amblyomma americanum* (Ixodidae). Exp. Appl. Acarol. *50*, 343–352.

9. Vermeire, J.J., Cho, Y., Lolis, E., Bucala, R., and Cappello, M. (2008). Orthologs of macrophage migration inhibitory factor from parasitic nematodes. Trends Parasitol. *24*, 355–363.
10. Dubreuil, G., Deleury, E., Crochard, D., Simon, J.-C., and Coustau, C. (2014). Diversification of MIF immune regulators in aphids: link with agonistic and antagonistic interactions. BMC Genomics *15*, 762.
11. Cho, Y., Jones, B.F., Vermeire, J.J., Leng, L., DiFedele, L., Harrison, L.M., Xiong, H., Kwong, Y.-K.A., Chen, Y., Bucala, R., et al. (2007). Structural and functional characterization of a secreted hookworm Macrophage Migration Inhibitory Factor (MIF) that interacts with the human MIF receptor CD74. J. Biol. Chem. *282*, 23447–23456.
12. Vandermoten, S., Harmel, N., Mazzucchelli, G., De Pauw, E., Haubruge, E., and Francis, F. (2014). Comparative analyses of salivary proteins from three aphid species. Insect Mol. Biol. *23*, 67–77.
13. Jaworski, D.C., Bowen, C.J., and Wasala, N.B. (2009). *Amblyomma americanum* (L): tick macrophage migration inhibitory factor peptide immunization lengthens lone star tick feeding intervals in vivo. Exp. Parasitol. *121*, 384–387.
14. Kamir, D., Zierow, S., Leng, L., Cho, Y., Diaz, Y., Griffith, J., McDonald, C., Merk, M., Mitchell, R.A., Trent, J., et al. (2008). A Leishmania ortholog of macrophage migration inhibitory factor modulates host macrophage responses. J. Immunol. *180*, 8250–8261.
15. Jackson, A.L., Bartz, S.R., Schelter, J., Kobayashi, S.V., Burchard, J., Mao, M., Li, B., Cavet, G., and Linsley, P.S. (2003). Expression profiling reveals off-target gene regulation by RNAi. Nat. Biotechnol. *21*, 635–637.
16. Tjallingii, W.F., and Gabryś, B. (1999). Anomalous stylet punctures of phloem sieve elements by aphids. Entomol. Exp. Appl. *91*, 97–103.
17. Gao, L.-L., Klingler, J.P., Anderson, J.P., Edwards, O.R., and Singh, K.B. (2008). Characterization of pea aphid resistance in *Medicago truncatula*. Plant Physiol. *146*, 996–1009.
18. Zheng, X., McLellan, H., Fraiture, M., Liu, X., Boevink, P.C., Gilroy, E.M., Chen, Y., Kandel, K., Sessa, G., Birch, P.R., and Brunner, F. (2014). Functionally redundant RXLR effectors from *Phytophthora infestans* act at different steps to suppress early flg22-triggered immunity. PLoS Pathog. *10*, e1004057.
19. Keller, H., Blein, J.P., Bonnet, P., and Ricci, P. (1996). Physiological and molecular characteristics of elicitin-induced systemic acquired resistance in tobacco. Plant Physiol. *110*, 365–376.
20. Lherminier, J., Benhamou, N., Larrue, J., Milat, M.L., Boudon-Padieu, E., Nicole, M., and Blein, J.P. (2003). Cytological characterization of elicitin-induced protection in tobacco plants infected by *Phytophthora parasitica* or *Phytoplasma*. Phytopathology *93*, 1308–1319.
21. Jung, H., Seong, H.-A., and Ha, H. (2008). Critical role of cysteine residue 81 of macrophage migration inhibitory factor (MIF) in MIF-induced inhibition of p53 activity. J. Biol. Chem. *283*, 20383–20396.
22. Kuśnierczyk, A., Winge, P., Jørstad, T.S., Troczyńska, J., Rossiter, J.T., and Bones, A.M. (2008). Towards global understanding of plant defence against aphids—timing and dynamics of early Arabidopsis defence responses to cabbage aphid (*Brevicoryne brassicae*) attack. Plant Cell Environ. *31*, 1097–1115.
23. Rodriguez, P.A., Stam, R., Warbroek, T., and Bos, J.I.B. (2014). Mp10 and Mp42 from the aphid species *Myzus persicae* trigger plant defenses in *Nicotiana benthamiana* through different activities. Mol. Plant Microbe Interact. *27*, 30–39.
24. Salminen, A., and Kaarniranta, K. (2011). Control of p53 and NF-κB signaling by WIP1 and MIF: role in cellular senescence and organismal aging. Cell. Signal. *23*, 747–752.
25. Lue, H., Kapurniotu, A., Fingerle-Rowson, G., Roger, T., Leng, L., Thiele, M., Calandra, T., Bucala, R., and Bernhagen, J. (2006). Rapid and transient activation of the ERK MAPK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on JAB1/CSN5 and Src kinase activity. Cell. Signal. *18*, 688–703.
26. Xu, L., Li, Y., Sun, H., Zhen, X., Qiao, C., Tian, S., and Hou, T. (2013). Current developments of macrophage migration inhibitory factor (MIF) inhibitors. Drug Discov. Today *18*, 592–600.
27. Baeza Garcia, A., Pierce, R.J., Gourbal, B., Werkmeister, E., Colinet, D., Reichhart, J.-M., Dissous, C., and Coustau, C. (2010). Involvement of the cytokine MIF in the snail host immune response to the parasite *Schistosoma mansoni*. PLoS Pathog. *6*, e1001115.
28. Panstruga, R., Baumgarten, K., and Bernhagen, J. (2015). Phylogeny and evolution of plant macrophage migration inhibitory factor/D-dopachrome tautomerase-like proteins. BMC Evol. Biol. *15*, 64.
29. Jaworski, D.C., Jasinskas, A., Metz, C.N., Bucala, R., and Barbour, A.G. (2001). Identification and characterization of a homologue of the pro-inflammatory cytokine Macrophage Migration Inhibitory Factor in the tick, *Amblyomma americanum*. Insect Mol. Biol. *10*, 323–331.